



APPENDIX A
LISTING OF CLAIMS

APPENDIX A: LISTING OF CLAIMS

Claim 3. A method for binding a DNA binding polypeptide of the Cys2 His2 zinc finger class to a DNA triplet in a target DNA sequence comprising 5-meC as the central residue in the target DNA triplet, the method comprising preparing a DNA binding polypeptide of the Cys2 His2 zinc finger class to bind to the DNA triplet, wherein binding to the 5-meC residue by an α -helical zinc finger DNA binding motif of the polypeptide is achieved by placing an Ala residue at position +3 of the α -helix of the zinc finger, and exposing the DNA binding polypeptide to the target DNA sequence, whereby the DNA binding polypeptide binds to the target DNA sequence.

Claim 4. A method for binding a DNA binding polypeptide of the Cys2 His2 zinc finger class to a DNA triplet in target DNA sequence comprising 5-meC, but not to an identical triplet comprising unmethylated C, the method comprising preparing a DNA binding polypeptide of the Cys2 His2 zinc finger class to bind to the triplet comprising 5-meC, wherein binding to each base of the triplet by an α -helical zinc finger DNA binding motif in the polypeptide is determined as follows:

- a) if the 5' base in the triplet is G, then position +6 in the α -helix is Arg or position ++2 is Asp, or position +6 in the α -helix is Arg and position ++2 is Asp;
- b) if the 5' base in the triplet is A, then position +6 in the α -helix is Gln or Glu and ++2 is not Asp;
- c) if the 5' base in the triplet is T, then position +6 in the α -helix is Ser or Thr and position ++2 is Asp; or position +6 is a hydrophobic amino acid other than Ala;
- d) if the 5' base in the triplet is C, then position +6 in the α -helix is any amino acid, provided that position ++2 in the α -helix is not Asp;
- e) position +3 in the α -helix is Ala;
- f) if the 3' base in the triplet is G, then position -1 in the α -helix is Arg;
- g) if the 3' base in the triplet is A, then position -1 in the α -helix is Gln and position +2 is Ala;
- h) if the 3' base in the triplet is T, then position -1 in the α -helix is Asn; or position -1 is Gln and position +2 is Ser;

APPENDIX A
LISTING OF CLAIMS

i) if the 3' base in the triplet is C, then position -1 in the α -helix is Asp and Position +1 is Arg; and

exposing the DNA binding polypeptide to the target DNA sequence, whereby the DNA binding polypeptide binds to the target DNA sequence.

³
Claim ~~23~~. The method according to claim ^{1 or 2} ~~3 or 4~~, wherein the binding protein comprises two or more zinc finger binding motifs.

⁴
Claim ~~25~~. The method according to claim ³ ~~23~~, wherein the DNA binding protein is constructed by recombinant DNA technology, the method comprising the steps of:

- a) preparing a DNA coding sequence encoding two or more zinc finger binding motifs;
- b) inserting the DNA sequence into a suitable expression vector; and
- c) expressing the DNA sequence in a host organism in order to obtain the DNA binding protein.

⁵
Claim ~~26~~. The method according to claim ^{1 or 2} ~~3 or 4~~ further comprising the steps of subjecting the DNA binding protein to one or more rounds of randomization and screening in order to improve the binding characteristics thereof.

⁶
Claim ~~28~~. The method of either of claims ^{1 or 2} ~~3 or 4~~, further comprising detecting the DNA binding polypeptide binding to the target DNA sequence.

⁷
Claim ~~29~~. The method of either of claims ^{1 or 2} ~~3 or 4~~, wherein the binding of the DNA binding polypeptide to the target DNA sequence regulates transcription of a gene.